Substrate Construes the Copper and Nickel Ions Impacts on the Mushroom Tyrosinase Activities

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Mushroom tyrosinase (MT) structural changes in the presence of Cu^{2+} and Ni^{2+} were studied separately. Far-UV CD spectra of the incubated MT with the either of the metal ions indicated reduction of the well-ordered secondary structure of the enzyme. Increasing in the maximum fluorescence emission of anilinonaphthalene-8-sulfonic acid (ANS) was also revealing partial unfolding caused by the conformational changes in the tertiary structure of MT. Thermodynamic studies on the chemical denaturation of MT by dodecyl trimethylammonium bromide (DTAB) showed decrease in the stability of MT in the presence of Cu^{2+} or Ni^{2+} using their activation concentrations. Both activities of MT were also assessed in the presence of different concentrations of these ions, separately, with various monophenols and their corresponding diphenols. Kinetic studies revealed that cresolase activity on *p*-coumaric acid was boosted in the presence of either of the metal ions, but inhibited when phenol, L-tyrosine, or 4-[(4-methylphenyl)azo]-phenol was substrate. Similarly, catecholase activity on caffeic acid was enhanced in the presence of Cu^{2+} or Ni^{2+}, but inhibited when catechol, L-DOPA, or 4-[(4-methylbenzo)azo]-1,2-benzenediol was substrate. Results of this study suggest that both cations make MT more fragile and less active. However, the effect of the substrate structure on the MT allosteric behavior can not be ignored.

Key Words : Mushroom tyrosinase, Monophenols, Diphenols, Nickel, Copper

Introduction

Tyrosinase (EC 1.14.18.1) is a bifunctional enzyme, which catalyzes ortho-hydroxylation of monophenols (cresolase activity) and oxidation of catechols to the corresponding ortho-quinones (catecholase activity).[1] *o*-Quinones follow some other enzymatic and nonenzymatic reactions, which result in formation of biopolymers like melanin. This macromolecule, the most famous product of tyrosinase, is the natural pigment of mammalian hair, eye, and skin.[2,3] Undesirable browning of fruits and vegetables during post-harvest handling has also been ascribed to tyrosinase.[4]

The interaction of protein with a metal ion is of interest because of various reasons: this includes biochemical standpoint and pathological prospective. Studying the interactions between metal ions and proteins are important especially with respect to their thermodynamic and kinetic stabilities.[5,6] Moreover, such studies could be informative about the enzymatic activity under conditions of metal ion tolerance and toxicity.[7]

Survey of literatures shows that the impact of metal ions on the melanogensis process have been studied from different but mainly nonenzymatic aspects such as cation exchange properties of melanins,[8,9] melanin structural modification induced by metal ions,[10] effect of metal ions on the rearrangement of dopachrome[11] etc. In contrast, less attention has been paid to the possible interactions of metal ions and tyrosinase. However, a characteristic feature of melanin-containing tissues is their high content of some heavy metals, in particular zinc, copper and iron.[12] For example, high level of these metal ions have been found in the choroid of eye,[13,14] black hair,[15] pigmented moles[16] and isolated melanosoms from Harding passey, horse and human melanomas.[17]

Similarities between mushroom tyrosinase (MT) and other tyrosinases, specially mammal tyrosinase,[18,19] has made it an apt example for in-vitro studies in this field. So, in pursuit of our works on MT stability[20-22] and considering the importance of the enzyme-divalent metal ion interactions,[23-27] this work was devoted to an investigation on the effect of Cu^{2-} and Ni^{2-} on the MT structure and activity. In a previous work on *Sepia officinalis* tyrosinase, it had been reported that Cu^{2-} inhibited tyrosinase activities while Ni^{2-} showed no effect.[28]

Materials and Methods

Materials. Mushroom tyrosinase (MT; EC 1.14.18.1), specific activity 3400 units/mg, was purchased from Sigma. Dihydrocaffeic acid, Caffeic acid (CA), *p*-coumaric acid (COA), phenol, catechole, L-tyrosine and 3-(3,4-dihydroxyphenyl) alanine (L-DOPA), were taken from authentic samples, and 4-[(4-methylphenyl) azo]-phenol (Me-PAPh) and 4-[(4-methylbenzo) azo]-1,2-benzenediol (MeBACat), were synthesized.[29] Dodecyl trimethylammonium bromide (DTAB) and anilinonaphthalene-8-sulfonic acid (ANS) were obtained from sigma. Analytical grade of $CuSO_4$ and $Ni(NO_3)_2$ from Merck were used. The buffer used in the assay was 2.5 mM phosphate buffer solution (PBS), pH = 6.8, which its salts were obtained from Merck. All experi-